UNITED STATES ENVIRONMENTAL
PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: Aug. 24, 2017

SUBJECT: Science Review of Human Study of Mosquito Repellent Performance of , containing Hydrogenated Catmint Oil (Refined Oil of *Nepeta cataria*) as their active.

Decision Number: 530133
DP Number: 441150
EPA File Symbol Number: 352-901
Chemical Class: Biochemical
PC Code: 004801
CAS Number: 8023-84-5
Active Ingredients: Hydrogenated Catmint Oil (Refined Oil of *Nepeta cataria)*
Tolerance Exemptions: Non-food
MRID Numbers: 501913-01

FROM: Clara Fuentes, Ph.D.
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THROUGH: Russell Jones, Ph.D., Senior Scientist.
Biochemical Pesticides Branch
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/s/ 08/31/2017

TO: Menyon Adams, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

RE: Carroll, S. (2017). Efficacy Test of Hydrogenated Catmint Oil (Refined Oil of *Nepeta cataria*) Against Mixed populations of Mosquitoes in the Field. Document dated February 16, 2017. Unpublished document prepared by Carroll-Loye Biological Research under Project Identification Number DPT-001 MRID 501913-01 (127 pp).

ACTION REQUESTED

Conduct a science review of a completed mosquito field study. Determine the adequacy of the methods employed and the scientific validity of the reported data. Evaluate and assess the period of time, in hours, that the tested products repel adult mosquitoes within the *Aedes, Anopheles and Culex*. These data are required by the EPA as a registration condition for the following products, containing Hydrogenated Catmint Oil (Refined Oil of *Nepeta cataria*) as their active ingredient:
Insect Repellent Liquid, Pump Spray (7% w/w *Nepeta cataria);*
Insect Repellent Liquid, Pump Spray (15 % w/w *Nepeta cataria);*
Insect Repellent Lotion, Repels Mosquitoes and Black Flies (7% w/w *Nepeta cataria),* and
Insect Repellent Lotion, Repels Mosquitoes and Black Flies (15% w/w *Nepeta cataria).*

STUDY OBJECTIVE

To determine the complete protection time (CPT) of each formulation when applied at a typical consumer dose against wild populations of mosquito species within the genera *Aedes, Anopheles and Culex.*

CONCLUSION

Based on the experimental results, hours of complete protection for each formulation were 2.64 hrs. for 7% spray and 2.45 hrs. for 15% spray formulations. Hours of protection for the 7% and 15 % lotion formulations were 3.65 hrs. and 5.31 hrs., respectively.

COMMENTS AND RECOMMENDATIONS

Efficacy study MRID 501913-01 "Efficacy Test of Hydrogenated Catmint Oil (Refined Oil of *Nepeta cataria*) Against Mixed populations of Mosquitoes in the Field." is unacceptable.

- Scientific aspects of the research were assessed in terms of the recommendations of the draft EPA Guidelines §810.3700, but the study protocol was not jointly reviewed by EPA and the Human Study Review Board (HSRB) prior to study initiation.

- The following deficiencies make the study results and determination of CPT unreliable for each formulation:

  Sample size is too small and the justification provided for sample size of 6 subjects per treatment doesn't reflect latest Agency's recommendations for power analysis calculation of sample size determination.

  The experiment lacks enough treatment replications. The study was conducted at one site only, which constitute only one trial or treatment replication. Exposing alternate forearms from same subject at a different testing time during the same day and at the same site is pseudoreplication, and no true replication, of the experiment.

SCIENCE REVIEW

The study protocol and Informed Consent Form were approved by IRB, Independent Investigational Review Board Inc, Plantation, FL. Study MRID 501913-01 was conducted in accordance with OECD Principles of Good Laboratory Practices as revised 1997, which are compatible with U.S. EPA FIFRA (40 CFR Part 160)

Study Objectives:

To determine the Complete Protection Time (CPT) of 4 mosquito repellent formulations containing Hydrogenated Catmint Oil (Refined Oil of *Nepeta cataria*) as their active ingredient against adult mosquitoes under field conditions. To establish the median protection time to first confirmed landing for each formulation under field conditions to support mosquito repellency claim on labels.

Materials & Methods:

Study locations:

Testing was conducted on public lands near Minden, NV. The side was a combination of grassy areas bordered by dense hedges of willows, growing in marshy depression, where spots of standing water were present. Environmental conditions were monitored at hourly intervals.

Study Dates:

Repellent product test was initiated on July 16, 2011, and completed on September 1st, 2011.

Repellents Tested:

Insect Repellent Liquid, Pump Spray (7% w/w *Nepeta cataria);*
Insect Repellent Liquid, Pump Spray (15 % w/w *Nepeta cataria);*
Insect Repellent Lotion, Repels Mosquitoes and Black Flies (7% w/w *Nepeta cataria),* and
Insect Repellent Lotion, Repels Mosquitoes and Black Flies (15% w/w *Nepeta cataria).*
Based on the dosimetry data, rate of applications were 2.5 mg/cm^2 for lotions, and 1.6 mg/cm2 for liquid spray formulations. Applications were made volumetrically by technicians and laboratory staff using 1 ml syringes and gloved fingertip to spread the material evenly.

Tested positive control/comparison repellent:

None

Untreated Control:

Two untreated control subjects (1 male and 1 female) monitored the ambient landing pressure at intervals of 5 minutes every 30 minutes throughout the test. There were no statistical comparisons to the untreated controls. Controls experienced 5 landings within 5 minutes, indicating the landing pressure was adequate throughout the study. Control subjects covered exposed skin as soon as 5 landings occurred within 5 minutes. Each untreated control subject was attended by 2 assistants who used aspirators for collecting landing mosquitoes.

Number of Test Subjects/Treatment Regime:

A total of 29 subjects were recruited, including 3 alternates, from a pool of 92 subjects. Twenty-six subjects were randomly selected to participate in the efficacy study. Sample size was 6 subjects (3 males and 3 females) per test material. Treated subjects exposed a repellent treated limb to mosquitoes continuously until product failure or cessation of the test. Technical personnel supervised exposures and assisted in aspirating mosquitoes as soon as they land on subjects. All landings were timed and recorded by time and subject.

Protocol used, including amendments:

Protocol DPT-001, "Efficacy Test of Hydrogenated Catmint Oil (Refined Oil of *Nepeta Cataria*) Against Mixed Populations of Mosquitoes in the Field" was used as amended on November 7, 2010. The amended protocol and Informed Consent can be found in Appendix 3 of the study.

Protocol Deviations:

No protocol deviations are reported in the study report.

Study Summary:

This mosquito repellent study was commissioned by E.I. DuPont de Nemours & Company to provide efficacy data for purposes of Pest Management Regulatory Agency (PMRA) of Health Canada registration. The test materials, containing active ingredient hydrogenated (refined) catmint oil (HCO) were Refined Oil of *Nepeta cataria* 7% Liquid (Insect Repellent Liquid, Pump-Spray), Refined Oil of *Nepeta cataria* 15% Liquid (Insect Repellent Liquid, Pump-Spray), Refined Oil of *Nepeta cataria* 7% Lotion (Insect Repellent Lotion, Repels Mosquitoes and Black Flies), and Refined Oil of *Nepeta cataria* 15% Lotion (Insect Repellent Lotion, Repels Mosquitoes and Black Flies). Data presented in this report doesn't include efficacy data on black flies.

The objective of the study was to determine the Complete Protection Time of each of the four HCO repellent formulations, when applied at a typical consumer dose, against field populations of mosquitoes of the genera Culex, Anopheles, and Aedes.

Margin of Exposure (MOE) values were calculated for the product formulations and found to be sufficiently large to justify dermal exposure of test subjects to the test materials during efficacy testing. The study Protocol was reviewed and approved by Independent Investigational Review Board, Inc., and by PMRA of Health Canada, but not jointly reviewed by EPA and HSRB prior to test initiation.

Repellent efficacy was assessed for each repellent formulation under field conditions. Three female and three male human subjects exposed a repellent-treated forearm to mosquitoes continuously until product failure or cessation of the test. Simultaneously, one male and one female untreated control subject exposed forearms or lower legs for up to 5 minutes every half hour in order to assess mosquito biting pressure. Both controls experienced at least five landings within five minutes of exposure throughout each test day, indicating that mosquito populations were suitably active for the efficacy study.

The mosquito species encountered at test site were *Aedes increpitus, Ae. dorsalis, Ae. cf.fitchii, Ae. nigromaculis, Aedes vexans, Culex tarsalis, Culiseta incidens*, and *Anopheles freeborni*. The reported mean Complete Protection Time for each repellent formulation were 2.64 hours

for the 15% lotion.

Experimental design:

Efficacy was tested in one habitat where West Nile Virus was not present for at least 2 weeks prior to testing. Collected mosquitoes were identified and pooled for viral detection assays employing the Polymerase Chain Reaction (PCR) methodology. Mosquitoes were assayed for West Nile Fever virus, Western Equine Encephalitis virus and St. Louis Encephalitis virus. Abiotic factors, temperature, wind speed, relative humidity and light intensity were recorded hourly. Six subjects each were randomly assigned to one of the 4 repellent treatments for a total of 6 subjects per treatment at one site. The sample size is 6 treated subjects per product per field trial. Repellent doses were prepared for each subject based on the surface area of lower legs and forearms of 26 subjects. A standard dosing rate of 2.5 mg/cm^2 for lotions and 1.6 mg/cm^2 for sprays were chosen to match those of previous efficacy studies with this active ingredient. Margin of exposure was estimated on standard dose of application and largest estimate of individual surface area to be treated, NOAEL of active ingredient (> 1,000 mg/kg from 28-day dermal toxicity in rat), percent concentration in each test material, standard 70 kg body weight (bw) of adult male, and a factor of 20% difference in dermal absorption between rats and humans. Predicted grams of active ingredient applied for 7% and 15% Lotions: 0.195g and 0.412 g, respectively. Predicted grams of active ingredient applied for 7% and 15% Spray formulations: 0.125g and 0.267g, respectively. Rate of application (mg/kg) in 70 kg bw = 2.79; 5.89; 1.79, and 3.81 mg/kg for Lotions 7 and 15% and Spray formulations 7% and 15 %, respectively. The corresponding **MOEs** for Lotions 7 and 15%, and Spray formulations 7% and 15 % are: 358 x (20% dermal absorption factor) = **7160**; 170 x 20% = **3400**; 559x20% = **11,180**, and 262x20% = **5,240**, respectively. In each case, the standard dose expressed as repellent weight per unit of skin surface area is converted to volume using the specific gravity of the test material. The amount of product applied to each subject is adjusted to their skin surface area so that the standard rate is applied to all subjects. Half the subjects on the test date were randomly treated on the right forearm and the other half on the left forearm with one of the 4 formulations. The test material was initially applied in advance to limit test duration to maximum hours of protection period. Those data were replaced with treatment to the other arm for what is referred as a second trial. The second time, products were applied without delayed period on alternate forearms of same subjects, and the previously treated arm was washed to remove treatment. In the field, treated subjects were arranged in pairs and equipped with aspirators to collect mosquitoes as soon as they land. Continuous exposure to mosquitoes consisted of 10 minutes breaks every hour. Untreated subjects exposed untreated arms for 5 minutes every 30 minutes or until 5 mosquitoes landed. All formulations were tested simultaneously the same day (July 16, 2011) at 2 different blocks of time (trials) during the same day. First trial began at 16:50 hours and ended at 18:20 hours. Second trial began at 19:50 hours and ended at 22:20 hours. For each trial, treatments were applied on alternate forearms of same subjects. There were 4 treatments with 6 subjects per treatment. Each treatment was applied blindly and randomly. All 6 subjects within the same treatment group was tested for only one formulation type during the study.

Data analysis:

Subjects remained in the test until the repellent failed as determined by the first confirmed landing, or until the end of the test period, whichever came first. The time at which the repellent failed equaled the Complete Protection Time (CPT), and a CPT was recorded for each subject. Collected data were analyzed by Kaplan-Meier survival analysis. Mean CPT for each repellent was calculated across all 6 subjects and reported as mean CPT + SD with the respective 95% confidence interval by fitting a Weibull distribution; Median CPT was also reported for each product treatment.

Results:

7% Liquid (Spray) Efficacy

Four of the six subjects testing 7% Liquid (spray) received confirming landings.

Table 1. Refined Oil of *Nepeta cataria* 7% Liquid (spray) efficacy: Weibull mean Complete Protection Times (CPTs) with lower and upper bounds of 95% confidence intervals.

Parameter	Parameter value[1]	Lower 95%	Upper 95%
Weibull mean	2.51	1.46	4.33
Normal mean[2]	1.82	0.74	2.90
Kaplan-Meier median	2.57	0.83	

[1]Parameters are computed from actual and estimated CPTs (for nonfailing subjects).
[2]Normal means are based on assigning the time of study termination as the time of failure for subjects that did not fail.

7% Lotion Efficacy

Four of the six subjects testing 7% Lotion received confirming landings.

Table 2. Refined Oil of *Nepeta cataria* 7% Lotion efficacy: Weibull mean Complete Protection Times (CPTs) with lower and upper bounds of 95% confidence intervals.

Parameter	Parameter value[1]	Lower 95%	Upper 95%
Weibull mean	3.73	2.15	6.47
Normal mean	2.92	2.16	3.68
Kaplan-Meier median	3.72	1.97	

[1]Parameters are computed from actual and estimated CPTs (for nonfailing subjects).

15% Liquid (Spray) Efficacy

Three of the six subjects testing 15% Liquid (spray) received confirming landings.

Table 3. Refined Oil of *Nepeta cataria* 15% Liquid (spray) efficacy: Weibull mean Complete Protection Times (CPTs) with lower and upper bounds of 95% confidence intervals.

Parameter	Parameter value[1]	Lower 95%	Upper 95%
Weibull mean	3.19	1.69	6.02
Normal mean	1.96	0.78	3.13
Kaplan-Meier median		0.33	

[1]Parameters are computed from actual and estimated CPTs (for nonfailing subjects).

15% Lotion Efficacy

Two of the six subjects testing 15% Lotion received confirming landings.

Table 4. Refined Oil of *Nepeta cataria* 15% Lotion efficacy: Weibull mean Complete Protection Times (CPTs) with lower and upper bounds of 95% confidence intervals.

Parameter	Parameter value[1]	Lower 95%	Upper 95%
Weibull mean	5.65	2.53	12.67
Normal mean[2]	2.92	1.70	4.14
Kaplan-Meier median	5.03	1.42	

[1]Parameters are computed from actual and estimated CPTs (for nonfailing subjects).

cc: *Clara Fuentes, RAL Menyon Adams*, BPPD Chron File, IHAD/ARS FT, PY-S: 08/24/2017